Genetic analysis of individual origins supports isolation of grizzly bears in the Greater Yellowstone Ecosystem

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Abstract: The Greater Yellowstone Ecosystem (GYE) supports the southernmost of the 2 largest remaining grizzly bear (*Ursus arctos*) populations in the contiguous United States. Since the mid-1980s, this population has increased in numbers and expanded in range. However, concerns for its long-term genetic health remain because of its presumed continued isolation. To test the power of genetic methods for detecting immigrants, we generated 16-locus microsatellite genotypes for 424 individual grizzly bears sampled in the GYE during 1983–2007. Genotyping success was high (90%) and varied by sample type, with poorest success (40%) for hair collected from mortalities found ≥1 day after death. Years of storage did not affect genotyping success. Observed heterozygosity was 0.60, with a mean of 5.2 alleles/marker. We used factorial correspondence analysis (Program GENETIX) and Bayesian clustering (Program STRUCTURE) to compare 424 GYE genotypes with 601 existing genotypes from grizzly bears sampled in the Northern Continental Divide Ecosystem (NCDE) (F_{ST} = 0.096 between GYE and NCDE). These methods correctly classified all sampled individuals to their population of origin, providing no evidence of natural movement between the GYE and NCDE. Analysis of 500 simulated first-generation crosses suggested that over 95% of such bears would also be detectable using our 16-locus data set. Our approach provides a practical method for detecting immigration in the GYE grizzly population. We discuss estimates for the proportion of the GYE population sampled and prospects for natural immigration into the GYE.

Key words: Bayesian clustering, DNA, factorial correspondence, grizzly bear, immigration, Northern Continental Divide, Yellowstone, *Ursus arctos*

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The abundance and range of grizzly bears (*Ursus arctos*) in the contiguous 48 states has declined during the 20th century. Grizzly bears were eliminated from 98% of their historic range (Mattson et al. 1995), and 31 of 37 bear populations recognized in 1922 were eliminated by 1975 (Servheen 1999). For grizzly bears in the Greater Yellowstone Ecosystem (GYE), the effect was one of increasing isolation. By 1959, when Craighead et al. (1995) began their pioneering work on grizzly bears in Yellowstone, the population had been reduced to a fraction of its former size and was relegated largely to Yellowstone National Park (YNP) and surrounding environs. High grizzly bear mortality in 1970 and 1971 following closure of open-pit dumps in YNP (National Academy of Sciences 1974) combined with uncertainty about population status prompted the US Fish and Wildlife Service (USFWS) in 1975 to list the species as threatened south of Canada under the Endangered Species Act (USFWS 1993). After listing, the population continued to decline (Knight and Eberhardt 1984, 1985, 1987) until strategies

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designed to reduce human-caused mortality were implemented in the mid-1980s (Interagency Grizzly Bear Committee 1986).

Evidence from 2 independent datasets indicates that grizzly bear numbers in the GYE have increased since the mid 1980s. Counts of unique females with cubs-of-the-year (unduplicated females as per Knight et al. 1995) have increased (Harris et al. 2007), and consistent with this trend, estimates of λ derived from radio-monitored bears also indicate a positive population trend (Eberhardt et al. 1994, Eberhardt 1995, Boyce et al. 2001, Harris et al. 2006). Concurrent with this population increase, bears have continued to expand their range (Blanchard et al. 1992; Schwartz et al. 2002, 2006a; Pyare et al. 2004).

However, the population remains isolated and concerns for its genetic health continue. Miller and Waits (2003) reported a slight decline in genetic diversity for Yellowstone bears since the early 20th century. They concluded that, given current population size, it was unlikely that genetic factors would negatively impact population viability in the near term. In the long term, they suggested that 1–2 migrants/generation from the Northern Continental Divide Ecosystem (NCDE) of northwest Montana (the geographically closest population) would maintain diversity.

Here we report on results of our initial efforts to determine if natural immigration into the GYE has occurred in recent years. We employed standard genetic techniques to analyze 16 microsatellite markers for a large number of grizzly bear samples obtained in the GYE. We used assignment tests and compared population-specific genotypes (Eldridge et al. 2001) from the GYE to those from the NCDE (Kendall et al. 2008, 2009) to identify the probable population of origin for individual bears. We also estimate our ability to detect immigrants in future monitoring based on our sampling intensity during 1983–2007.

Study area

We considered the landscape supporting grizzly bear populations in the GYE and the NCDE our study areas (Fig. 1). The GYE encompassed Yellowstone and Grand Teton National Parks (GTNP) plus portions of 6 adjacent national forests (Beaverhead–Deerlodge, Bridger–Teton, Caribou–Targhee, Custer, Gallatin, and Shoshone) and smaller amounts of state and private land in Wyoming, Montana, and Idaho, USA. Geographically, the GYE included headwaters of the Missouri–Mississippi, Snake–Columbia, and Green–Colorado river systems, the Yellowstone Plateau, and 14 surrounding mountain ranges (Marston and Anderson 1991). Detailed descriptions can be found in Blanchard and Knight (1991), Mattson et al. (1991), and Schwartz et al. (2006b).

The NCDE, located in the northern Rocky Mountains of Montana, included Glacier National Park, portions of 5 national forests (Flathead, Kootenai, Lewis and Clark, Lolo, and Helena), and 2 Indian Reservations (Blackfeet Nations, and Confederated Salish and Kootenai Tribes). Geographically, the NCDE encompassed 9 mountain ranges and included watersheds of the Flathead–Columbia, Missouri–Mississippi, and St. Mary–Saskatchewan river systems. Climates and associated vegetation varied, with distinctive Pacific maritime influences in the northwest and continental influences in the southeast (Daubenmire 1969). Climatically distinct portions of NCDE with associated key grizzly bear foods were described in Mace and Jonkel (1986).

During 1990–2004, grizzly bears in the GYE occupied roughly 37,000 km^2, and evidence suggested continued range expansion (Schwartz et al. 2006b). Occasional occurrences, usually in the form of conflicts or mortalities, were documented well beyond the area considered occupied (Interagency Grizzly Bear Study Team [IGBST] annual reports at http://nrmsc.usgs.gov/products/IGBST). Kendall et al. (2009) concluded that grizzly bears in the NCDE were also expanding their range and estimated the population occupied 33,480 km^2 during 1994–2007. Using these most recent estimates of occupied range (Schwartz et al. 2006b, Kendall et al. 2009) suggests that a straight-line distance of approximately 165 km separated the GYE from the NCDE population to the north (Fig. 1). Several major transportation corridors, including the east–west Interstate Highway 90 and the north–south Interstate Highway 15, occurred in the intervening terrain (Fig. 1). These routes generally follow broad valley bottoms in which livestock husbandry was the predominant activity.

Methods

GYE samples

Samples for genotyping were collected by bear researchers and managers from live captures and

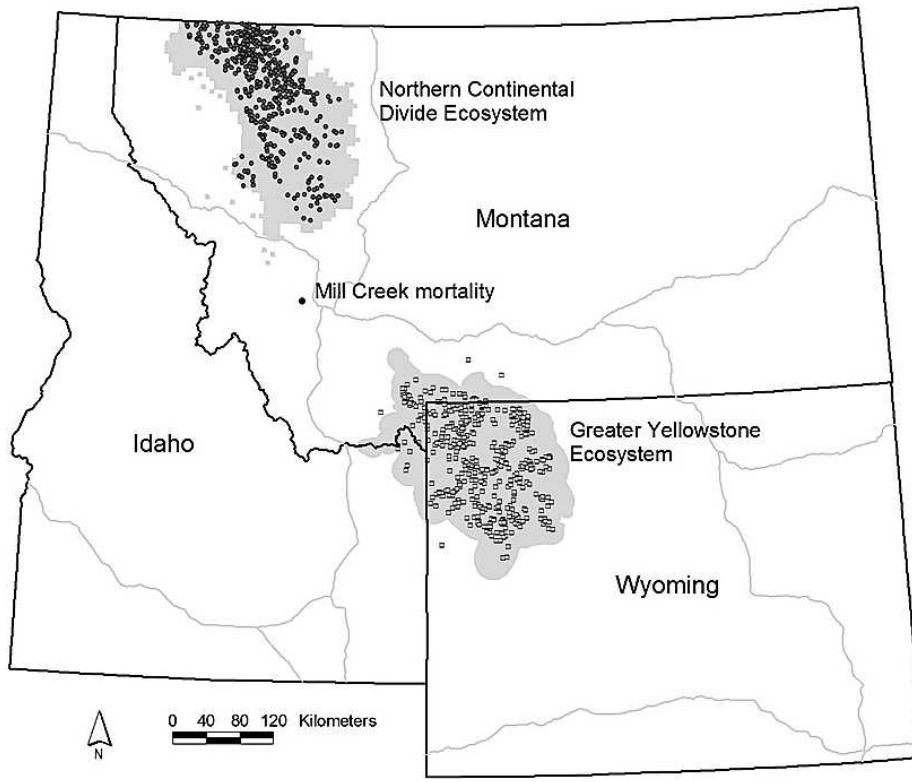

Fig. 1. The Greater Yellowstone (GYE) and Northern Continental Divide Ecosystems (NCDE), Idaho, Montana, and Wyoming, USA. Shaded areas are estimated current range for each grizzly bear population. Average locations for individual genotypes were estimated from geographic means of VHF telemetry locations (when available) in the GYE, and geographic means of location for hair collections for individuals in the NCDE. Gray lines show interstate highways.

mortalities throughout the GYE. Capture methods typically employed culvert traps or Aldrich leg-hold snares and are described in Schwartz et al. (2006b). A small number of hair samples ($n = 18$) were obtained using hair corrals where bears were not handled. These samples were obtained from an investigation of a suspected adoption by a female grizzly with cubs in YNP during 2007 (Haroldson et al. 2008a).

Type of sample materials varied over the duration of study but generally included hair, tissue, or blood. We placed hair samples in manila envelopes during handling and stored them in file cabinets affixed to original field forms at room temperature. We froze ear plugs at -32°C. We stored blood samples collected prior to 1996 as clots with ethylene glycol at -32°C. After 1997, we stored clots and some blood samples in Longmire lysis buffer solution (Longmire et al. 1988) at room temperature. The Wyoming Game and Fish Wildlife Forensic and Fish Health Laboratory also provided DNA material ($n = 53$) extracted from samples using standard phenol:chloroform and Eppendorf PhaseLock tubes or Super Quick Gene (ATCG, Fort Collins, Colorado, USA). They stored extracted samples in TE (Tris buffer + Ethylene diamine tetraacetic acid) buffer at -70°C (D. Hawk, Wyoming Game and Fish Department, Laramie, Wyoming, USA, personal communication, 2008).

Some samples were collected before genetic methods were widely available. Thus, no special consideration for genetic methodology was employed when samples, particularly hair samples, were originally archived. We used this as an opportunity to investigate the effect of storage time (in years) on genotyping success of hair samples. We used binary logistic regression (MiniTab version 14.20) to test genotyping success as a function of storage time in years.

Genetic methods. We used material from the most recent handling for known individuals with ≥1 capture (i.e., marked). After the initial sample selection, we made a random draw of 10 samples without replacement from individuals with multiple samples as blind tests for individual identification. We extracted DNA using DNeasy Tissue kits following manufacturer's instructions (Qiagen Inc., Mississauga, Ontario, Canada, http://www.qiagen.com/) at a laboratory (Wildlife Genetics International, Nelson, British Columbia, Canada) specializing in the analysis of low quantity and quality DNA material (Woods et al. 1999, Paetkau 2003, Roon et al. 2005). We genotyped successful DNA extractions for individuals at 16 well documented microsatellite sites (Ostrander et al. 1993, Taberlet et al. 1997, Paetkau et al. 1998, Proctor et al. 2002) used by Kendall et al. (2008, 2009): G1A, G10B, G10C, G1D, G10H, G10J, G10L, G10M, G10P, G10U, G10X, MU23, MU50, MU59, CXX20, and CXX110. Genotyping was completed by analyzing markers in 3 groups. Samples were considered unacceptably error-prone and culled from subsequent analysis if they failed at >5 of 11 markers during the first round of genotyping (Paetkau 2003). After the first group of markers was analyzed, individual identification was established based on a minimum of 9 markers for which all samples had complete genotypes. We prevented the identification of false individuals (genotyping error) by re-analyzing mismatching markers for pairs of genotypes that matched at 7 or 8 of 9 markers, an approach that was validated through extensive blind testing during the NCDE analysis (Kendall et al. 2009). Only 1 sample per individual had its genotype extended to the full set of 16 markers.

NCDE samples

Samples from the NCDE to investigate immigration into the GYE were from grizzly bear hair collected and analyzed as part of the Greater Glacier DNA study (Kendall et al. 2008) or the NCDE DNA effort (Kendall et al. 2009). Combined results from these studies produced 601 individuals with complete genotypes at the 16 microsatellite markers. During September 2005, a subadult male grizzly bear was found dead in Mill Creek, southwest of Anaconda, Montana, approximately 80 km south of the estimated distribution for NCDE bears, and 120 km northwest of the distribution of GYE bears. Montana Fish, Wildlife and Parks provided a 15-locus genotype for this bear that we included in our analysis.

Statistical analysis

We estimated variability within populations, differentiation between populations, and the significance of departures from Hardy-Weinberg equilibrium using Genepop 4.0.10 as implemented at "Genepop on the Web" (Rousset 2008; http://genepop.curtin.edu.au/). We used a multidimensional factorial correspondence analysis (FCA) within Program GENETIX (Belkhir 1999) to identify clusters of individuals with similar genotypes. The procedure provides an objective method for determining the birth population by making no a priori assumption of group membership. Individuals are grouped on multiple factorial axes based on shared alleles. We also used the Bayesian clustering Program STRUCTURE (Pritchard et al. 2000) to look for evidence of genetic exchange between the 2 populations. Program STRUCTURE uses a Markov chain Monte Carlo (MCMC) process to subdivide data into likely populations, then estimates the proportion of each individual's ancestry from each population. We used the admixture model with 1 million MCMC steps (including a 250,000-step burn-in period) and with k (the putative number of populations) set to 2. STRUCTURE is often used to estimate the number of populations in a dataset by exploring a range of values of k, but because our goal was to separate GYE bears from non-GYE bears, we did not explore higher values of k.

We also tested the power of STRUCTURE to detect first-generation hybrids by simulating 500 hybrid genotypes by drawing 1 allele at random from the allele distributions observed in the GYE and NCDE datasets using a random number function in Microsoft Excel. This process was repeated for each marker to create 16-locus genotypes with 1 allele from each population at each locus. Drawing alleles at random should provide a reasonable reflection of detection power in a system with no recent history of immigration, but if immigrants enter the Yellowstone population, it will become necessary to employ more sophisticated sampling methods that account for gametic disequilibrium (Paetkau et al. 2004).

Hybrid genotypes were analyzed by adding them in sets of 10 to the original STRUCTURE input file containing data from the 424 + 601 actual bears, based on the observation that larger additions of hybrid genotypes reduced the power of the program

to apportion the ancestry of non-hybrid (real) individuals.

Probability of detection. We estimated the cumulative probability of detecting a new migrant to the GYE population over time using a given amount of search effort. First, we estimated the proportion of the population genotyped in 2007. We derived this proportion by summing new captures, mortalities, and bears known to be alive because of radiotelemetry in 2007. These 122 samples represented 21% of the total standing population estimated that year (571; Haroldson 2008).

We further estimated the number of new individuals that would become available for genotyping after 2007 if we continue to sample at our current rate. For 1998–2007, the IGBST, in conjunction with state wildlife management agencies (Idaho, Montana, Wyoming) and National Park Service managers (GTNP, YNP), annually trapped and handled 36 new individuals (IGBST annual reports, http://nrmsc.usgs.gov/products/IGBST) and documented an average of 9 mortalities from unmarked individuals. Thus, on average, we sampled approximately 45 new independent bears (≥2 years of age) each year. We assumed the population would continue to grow at current rates (estimated point $\lambda = 1.04$, Harris et al. 2006) and that survival for independent bears were similar to estimates (male = 0.874, female = 0.95) by Haroldson et al. (2006). We also assumed we would only sample independent bears because the IGBST rarely captured dependent young. We used Program RISKMAN (Taylor et al. 2001) with demographic parameters for the GYE population to estimate the proportion of dependent young (0.249), independent males (0.294), and independent females (0.458). In 2007, we estimated there were 142 dependent young (571 x 0.249) and 429 independent bears (male = 168, and female = 261). Therefore, we estimated there were 307 independent bears that had not been genotyped (429 - 122). Thus, we estimated the probability of detecting 1 new migrant in 2007 as 0.146 (45/307). Carrying these calculations forward, we applied the estimated λ to the estimated population size and reduced the number of known marked bears by the weighted mortality rate for independent male and female bears in the population. We then estimated the probability of detecting a new individual in 2008 and subsequent years. We estimated the annual cumulative probability of detecting a migrant entering the population in 2007 using the formula:

$$1-\left(\prod_{t=1}^{n}(1-X_t)\right)$$

where t = year, beginning in 2007, and
X_t = the annual probability of detecting a new individual (adapted from McArdle 1990).

Results

GYE genotyping

We successfully genotyped 451 of 496 samples. Accounting for samples with identical multilocus genotypes (assumed to come from the same individual), our final dataset contained complete 16-locus genotypes for 424 unique individuals. Genotyping success varied by sample type (Table 1), with hair samples having the lowest success rate (90%) and whole blood or tissue samples having the highest (100%). Among different sources of hair, the largest percent of failures (40%) were from dead bears when samples were obtained >1 day after death (Table 1).

Samples were obtained from 1983–2007, with the largest number (n = 86, 17%) coming from 2007 because we typically used the most recent sample available from each bear. Among hair samples from captured bears with adequate material (Table 1, n = 294), there was no evidence that the number of years samples were stored (range = 0–24 yrs) affected genotyping success (G = 2.532, 1 df, P = 0.112). This suggested that other factors, possibly condition of samples at the time of archiving, had a greater effect on genotyping success than storage time.

We included 10 samples (2%) from randomly chosen individuals who had been captured more than once as blind tests for individual identification. Of these, 9 were correctly identified as duplicated samples and 1 pair failed due to poor quality. We also successfully resolved the identities of 4 individuals with lost or inconclusive marks (lost tags or unreadable tattoos) at handling. In each instance, differences in estimated ages (from cementum annuli count of sectioned teeth, Stoneberg and Jonkel 1966) between initial capture and subsequent capture were consistent with the genetic results identifying the samples as having come from a single individual. For example, we captured bear 204 at age 2 in 1992 and a 17-year-old bear 15 years later in 2007 with no marks (designated 575). Genotyping revealed that 575 and 204 were identical; their age difference was consistent with the number of years between sampling.

Table 1. Grizzly bear samples analyzed and successfully genotyped by sample origin and type, Greater Yellowstone Ecosystem, 1983–2007.

			Genotyping result				
Sample origin	**Sample type**	**Total**	**Successful (%)**	**Failed**	**Inadequate[a]**	**Mixed[b]**	**Black bear**
Sampled at capture	hair	298	268 (89.9)	26	3	1	0
	blood (buffered)	53	53 (100.0)	0	0	0	0
	blood (EDTA)[c]	2	2 (100.0)	0	0	0	0
	blood (frozen clot)	46	45 (97.8)	1	0	0	0
	tissue (ear plug)	4	4 (100.0)	0	0	0	0
	extracted DNA	53	49 (92.5)	4	0	0	0
Hair snag	hair	20	17 (85.0)	1	0	1	1
Found mortality sampled	hair	9	8 (88.9)	1	0	0	0
≤1 day after death	tissue (muscle)	1	1 (100.0)	0	0	0	0
Found mortality sampled							
>1 day after death	hair	10	4 (40.0)	5	1	0	0
Total		496	451 (90.9)	38	4	2	1

[a]Sample lacked suitable material for extraction.
[b]Sample contained material from multiple bears.
[c]Blood preserved in ethylene diamine tetraacetic acid (EDTA).

Forty-six samples were replicated because they represented 2–8 samples from 19 individuals. These samples yielded a total of 628 single-locus genotypes (not all samples were genotyped at all 16 markers) for comparison. We detected a single error, giving an estimated error rate of 0.0016 (1/628). This error rate/sample/locus multiplied by the number of genotypes that were not replicated as positive controls (~390) suggested approximately 10 errors in the dataset. Thus, we expect that roughly 1 in 40 individuals might have contained an error at 1 of the 16 markers. This rate of error is not expected to have meaningful effects on analysis of individual origins. We expected the error rate in the 601 genotypes from the NCDE to be lower yet because those 16-locus genotypes were run in duplicate (Kendall et al. 2009). Kendall et al. (2009) reported an error rate of 0.002 for all loci/sample.

Because most of the GYE samples genotyped were from marked (i.e., handled) individuals, the chance of assigning samples to the same individual when they were in fact different bears was small. Still, we evaluated this potential for false matches using the distribution of genotype similarity (''mismatch distribution'', i.e., 1MM-pairs differ at 1 marker, 2MM-pairs differ at 2 markers, Paetkau 2003). We checked for matching genotypes before running all 16 markers, so we conservatively based our mismatch distribution on only the 9 markers for which every sample was analyzed. Our graphed mismatch distribution (Fig. 2) indicated 12 2MM-pairs (matches at 7 of 9 markers) among 424 individuals. By extrapolating the curve, we would not expect to observe any 1 MM-pairs, and this was true — there were no matches at 8 of 9 markers. Matches at all 9 markers would be even less common, suggesting little risk that we sampled 2 individual bears with the same 9-locus genotype.

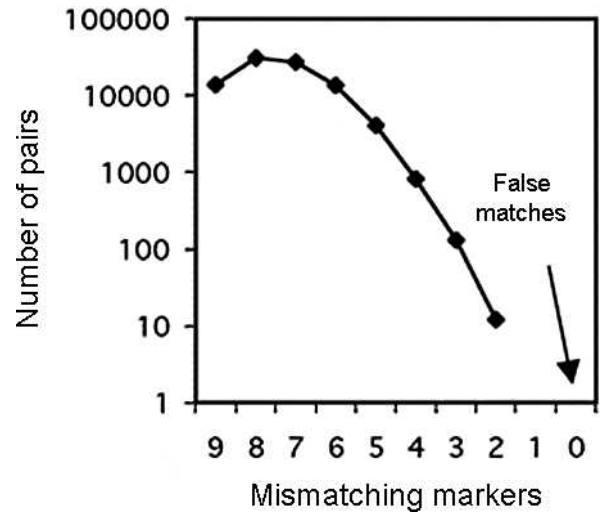

Fig. 2. Mismatch distribution for 424 grizzly bears in the Greater Yellowstone Ecosystem, 1983–2007, based on 9 markers that were used for establishing individual identities. Extrapolation suggests no realistic chance of different bears having the same genotype at 9 or more loci.

Table 2. Marker variability expressed as expected (H_E) and observed (H_O) heterozygosity, and observed number of alleles (A) for 16 microsatellite loci from 424 individual grizzly bears from the Greater Yellowstone Ecosystem, 1983–2007.

Locus	H_E	H_O	A
G1A	0.66	0.63	6
G1D	0.80	0.79	7
G10B	0.72	0.75	6
G10C	0.46	0.44	5
G10H	0.57	0.60	6
G10J	0.64	0.64	4
G10L	0.40	0.44	2
G10M	0.65	0.64	6
G10P	0.74	0.76	5
G10U	0.63	0.65	5
G10X	0.08	0.08	2
MU50	0.60	0.61	6
MU59	0.75	0.75	7
CXX20	0.52	0.48	3
CXX110	0.57	0.57	6
MU23	0.81	0.81	7
Mean	0.60	0.60	5.2

Genetic diversity and divergence

Our estimates of observed and expected heterozygosity using 16 markers for 424 genotyped bears were 0.60 and 0.60 (NCDE = 0.67 and 0.68), respectively (Table 2). We tested for heterozygote deficit at each marker, with one marker returning $P = 0.03$. One result with $P < 0.05$ in 16 tests is expected given our nominal Type I error rate, although a small heterozygote deficit would be anticipated in the likely event that the study population was not perfectly panmictic across the ~37,000 km^2 study area. Tests for linkage disequilibrium between each of 120 pairs of markers returned 48 with $P < 0.05$, which was inconsistent with our null hypothesis. This appeared to support the (biologically plausible) hypothesis that the GYE grizzly bear population was not completely panmictic.

We found a mean of 5.2 alleles/marker (compared with 7.5 in the NCDE). Marker G10X was essentially fixed in GYE bears ($H = 0.08$), but we included it because it is useful in differentiating GYE and NCDE populations. Estimated genetic distance F_{ST} between the GYE and NCDE was 0.096, similar to the highest values (0.09) described between regions of the NCDE (Kendall et al. 2009).

Assignment tests

FCA analysis for 424 individuals from the GYE and 601 NCDE bears produced non-overlapping value ranges for the 2 populations on the first axis (Fig. 3), providing strong evidence that no sampled individuals had moved between these populations. In addition, our FCA results (Fig. 3) indicated that the bear found dead in Mill Creek during 2005 was genetically similar to NCDE bears. Program STRUCTURE also produced strong separation between GYE and NCDE bears, with all NCDE bears having <21% of their estimated genetic ancestry in the GYE, and all GYE bears having >82% estimated ancestry in that population. Results were stable between runs, with the estimated proportion of any individual's ancestry in the GYE varying by <0.004 across 3 runs of STRUCTURE. Given the wide separation between GYE and NCDE bears with STRUCTURE (no values between 0.21 and 0.82 GYE ancestry) compared to the near overlap of clusters with the FCA analysis (Fig. 3), we used STRUCTURE to explore the potential for identifying F1 hybrids.

When the 500 simulated hybrid genotypes were analyzed in Program STRUCTURE, only 21 had estimated ancestry within the range observed for actual GYE bears, suggesting that approximately 96% (1–21/500) of first-generation hybrid offspring would be detectable by virtue of having values outside the observed range of GYE bears.

Probability of detection

Our estimated cumulative probability suggested that if a single migrant entered the GYE population, our probability of detection given our sampling intensity would have been about 0.147 in the year of immigration, but by 5 years later that probability would have increased to 0.589 (Fig. 4). The probability of detecting a first-generation hybrid during its initial year of independence (age 2) was approximately 0.141 (0.147 x 0.96), and 0.565 after 5 more years if it survived (Fig. 4).

Discussion

Overall, our genotyping success for GYE samples was high (90%) and varied by sample type. The poorest success (40%) was obtained using hair collected from dead bears found >1 day after death. This leads us to suggest that material other than hair should be collected from dead bears for genotyping, especially if the carcass shows any sign indicating time of death was >1 day prior to discovery. Good genotyping success using foot pads from dead bears have been achieved (Wildlife Genetics International,

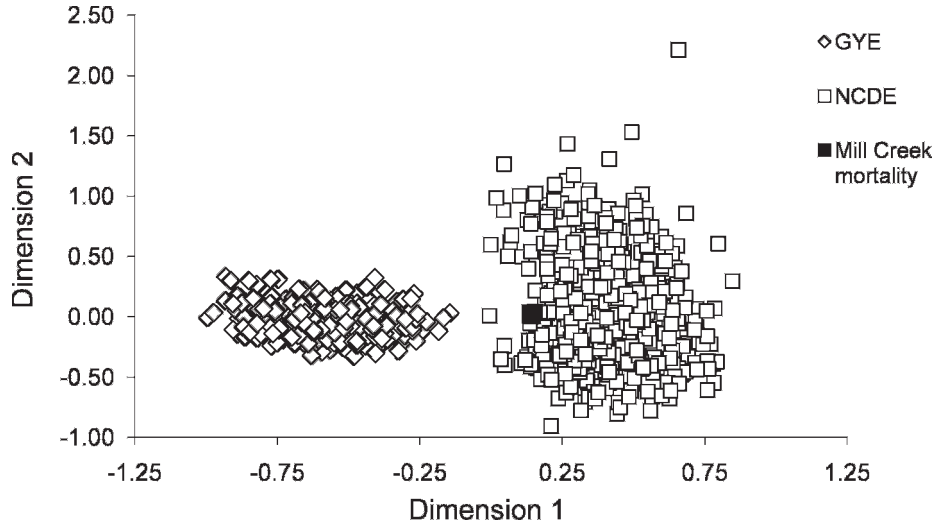

Fig. 3. Dimensions 1 and 2 Genetrix output comparing individual genotypes for grizzly bears from the Greater Yellowstone (GYE) and Northern Continental Divide Ecosystems (NCDE).

Nelson, British Columbia, Canada, unpublished data), and we suggest use of this tissue for bears found >1 day after death. We found that the length of time (in years) hair samples were stored was not a factor in genotyping success. Condition of the hair samples when initially stored likely had more impact on later genotyping success than time stored. Estimates for heterozygosity and allele frequencies we obtained for GYE grizzly bears were similar to previously published estimates (Paetkau et al. 1998, Waits et al. 1998, Miller and Waits 2003).

We found no evidence of recent immigrants from the NCDE or their first-generation progeny in GYE. Our results provide clear evidence that the techniques we employed would have identified migrants and most of their F1 offspring had we sampled them. The detection of progeny would indicate functional connectivity (immigration and breeding) between the GYE and other grizzly bear populations. The use of additional markers in the analysis or the passage of time in the absence of gene flow (i.e., drift) would both improve the ability to identify the genetic origin

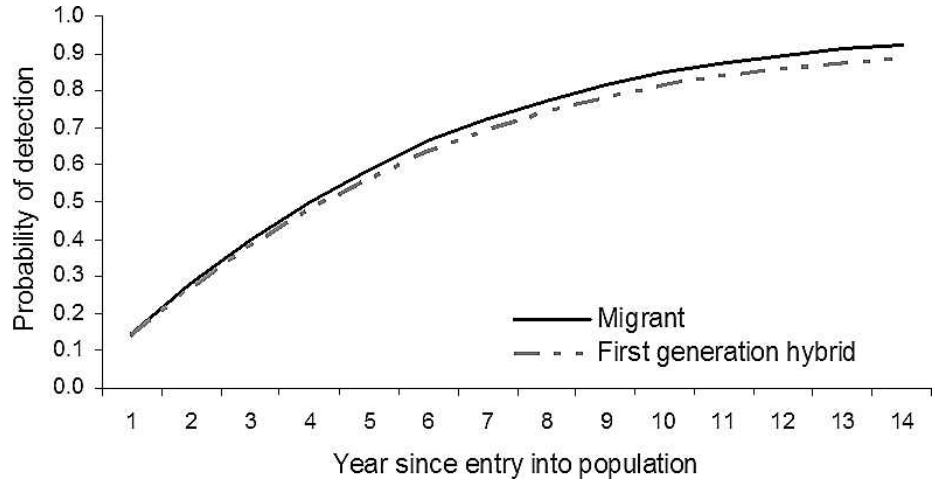

Fig. 4. Cumulative probability of detecting a single new migrant or first-generation hybrid in the Greater Yellowstone Ecosystem assuming 45 new individuals genotyped annually and assuming that the migrant entered the population (or a hybrid juvenile >2 years old became an independent bear) in year 1.

of first-generation offspring. Similar methods using 15 microsatellite markers were used to determine that a grizzly bear caught in the Canadian Flathead Valley (NCDE) in 1996 was likely a first-generation hybrid from NCDE and GYE parents (Wildlife Genetics International, Nelson, British Columbia, Canada, unpublished data). We know of 4 nuisance bears, 1 of which was a female accompanied by 2 cubs, that were transplanted to the Canadian Flathead from the GYE during 1982–83 (Knight and Blanchard 1983, Knight et al. 1985). Fates for 2 of these bears after transport were unknown. It is possible that 1 of these individuals survived and produced the putative hybrid caught in the Canadian Flathead. Additional support comes from captive grizzly bears at Washington State University (WSU). Fortin (2009) reported correct genetic identification by the laboratory that we used of 1 NCDE bear and 2 offspring from a NCDE and GYE mating that were included as blind tests among samples of YNP grizzly bear hair analyzed for a separate study.

Given near certainty that we can identify an immigrant or its progeny from the NCDE or other populations (Proctor et al. in press) if sampled, the probability of detection is a function of the prevalence in the GYE of grizzlies originating in other populations. Our cumulative estimates of detection (Fig. 4) assume constant population growth and mortality rates. If growth rate slows and we continue to mark the same number of individuals, our probability of detection would increase unless mortality rates also increase and we obtain fewer samples from bears that die. Our cumulative estimate of detection also assumes equal probability of capture among sex–age classes of bears, which is unlikely. We know from trapping records that subadult males are typically more vulnerable to capture than other sex–age classes of bears. We anticipate that a new migrant from the NCDE would likely be a subadult male, making our estimate of detection conservative. Finally, should a new migrant breed with a GYE resident, the probability of detecting a first-generation hybrid would be high (0.96), but likely delayed by 3 years until the offspring becomes independent because we sample so few cubs and yearlings. We therefore consider our sampling and genotyping effort sufficient to have a high probability of detecting natural immigrants or their progeny.

We were not surprised that we did not detect any immigrants from the NCDE. Studies involving marked and radio instrumented grizzly bears have been conducted almost continuously in the GYE since 1959 (Craighead et al. 1995, Haroldson et al. 2008b) and since the mid-1970s in the NCDE (Jonkel 1982). In nearly 50 years of combined effort involving hundreds of marked bears, natural movement between the GYE and other populations has not been documented. This suggests that the distance and the intervening terrain are significant barriers to movement between these populations, even for a highly vagile species such as the grizzly bear.

Natural connectivity between the NCDE and GYE requires corridors or linkage zones which at the minimum allow for some movement of individuals between these populations. Walker and Craighead (1997) used least-cost-path modeling to identify 3 potential corridors linking the NCDE grizzly population to the GYE. These were through (1) the Big Belt–Bridger–Gallatin mountain ranges, (2) the Boulder–Tobacco Root–Gravelly–Taylor–Hilgard ranges, and (3) the Selway–Bitterroot–Lemhi–Centennial–Madison ranges. Recently Cushman et al. (2009) produced similar results, identifying corridors 1 and 2 of Walker and Craighead (1997) using least-cost-path modeling and a genetically-based landscape resistance model for black bear (*Ursus americanus*). Cushman et al. (2009) suggested their black bear model was useful for predicting movement corridors for grizzly bears because of the similarity of their results with those of other studies of grizzly bear linkage (Sandstrom 1996, Servheen et al. 2001). Forest cover and road densities were important themes in the development of resistance layers for models of both Walker and Craighead (1997) and Cushman et al. (2009). Thus it is not surprising they identified similar potential linkage routes. Cushman et al. (2009:373, Table 1) also identified land ownership and potential barriers to animal movements along their predicted routes.

Characteristics of grizzly bear dispersal are additional factors that will influence the potential for natural immigration into the GYE. Grizzly bear dispersal is usually sex-biased (Blanchard and Knight 1991, McLellan and Hovey 2001, Proctor et al. 2004, Zedrosser et al. 2007), with males dispersing on average 3 times further than females (McLellan and Hovey 2001, Proctor et al. 2004). Mean male dispersal distances were 42 km and 70 km in southwestern Canada and the GYE, respectively (Blanchard and Knight 1991, Proctor et al. 2004), and dispersal distances of up to 175 km have been

documented (Proctor et al. 2004). Male grizzly bears are capable of more extreme movements. In the Canadian Arctic a male bear was observed on Melville Island, Northwest Territories, >600 km from the mainland where the nearest female grizzly would likely reside (Doupe et al. 2007). A result of this bias in dispersal and movements is an asymmetric susceptibility to fragmentation between the sexes (Proctor et al. 2004, 2005). However, the possibility of connectivity by at least male bears is the minimum requirement to maintain the genetic health of the GYE population. The ~165 km distance between occupied ranges for these populations is 2–3 times the mean dispersal distance, but approximately equal to the maximum distances observed for male bears in the region.

Kendall et al. (2009) suggested that the NCDE population expanded its range in recent years. If this trend continues, suitable habitats south of the current NCDE population along several possible linkage zones may become reoccupied. Although trends in range expansion in the GYE have been more southerly (Pyare et al. 2004, Schwartz et al. 2006a, IGBST unpublished data), there is suitable habitat to the north and west of their current range that bears in the GYE continue to reoccupy. If the distance between the NCDE and GYE populations decreases, the potential for natural immigration to the GYE increases. The most likely scenario for such an event would involve a young male bear dispersing from recently reoccupied habitat across 1 or more transportation corridors. Supporting this hypothesis, the subadult male bear found dead in September 2005 in Mill Creek southwest of Anaconda, Montana, was approximately 80 km south of the estimated distribution for NCDE bears. This individual had NCDE ancestry; we believe it was probably dispersing because the Pioneer Mountains are not known to contain a resident population of grizzly bears. In a similar result, Proctor et al. (in press) reported that a grizzly bear killed by a black bear hunter in the Bitterroot Mountains of Idaho during the fall of 2007 likely originated in the Selkirk Mountains of southern British Columbia and thus had dispersed >240 km. The Bitterroot Mountains are also not known to contain a resident grizzly bear population.

Considering current trends in range expansion and population increases, the potential for natural immigration, at least by male bears, into the GYE has likely increased. However, increases in human development along potential linkage zones may negate this increased potential unless secure travel corridors are maintained among populations.

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